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Supplemental Material

Effects of Inorganic Arsenic, Methylated Arsenicals, and Arsenobetaine on Atherosclerosis in the apoE$^{-/-}$ Mouse Model and the Role of As3mt-Mediated Methylation

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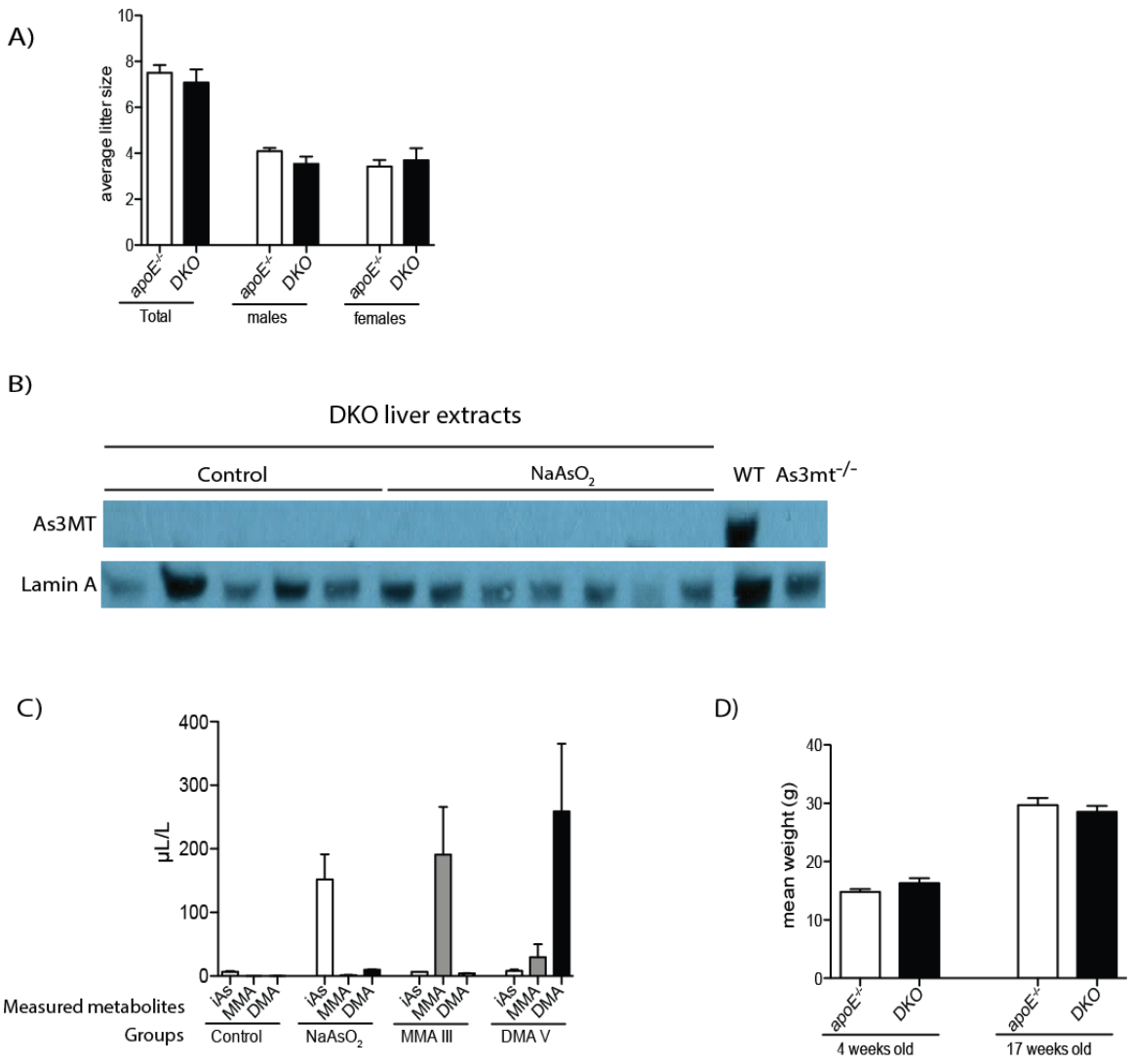

Figure S1: DKO model characterization and validation.

The litter size and sex ratio was similar in at least 12 mating pairs of apoE$^{-/-}$ and DKO mice (A). As3MT immunoblot of liver extracts from DKO mice (control and $NaAsO_2$-exposed), wild-type C57BL6, and As3mt$^{-/-}$ mice (B). Results from arsenic speciation by HPLC/ICP-MS in urine samples from DKO mice exposed to $NaAsO_2$, MMA III, DMA V, or tap water (C). DKO mice were unable to significantly metabolize arsenicals. Unexposed mice from both genotypes had similar weight at 4 weeks and 17 weeks (D).

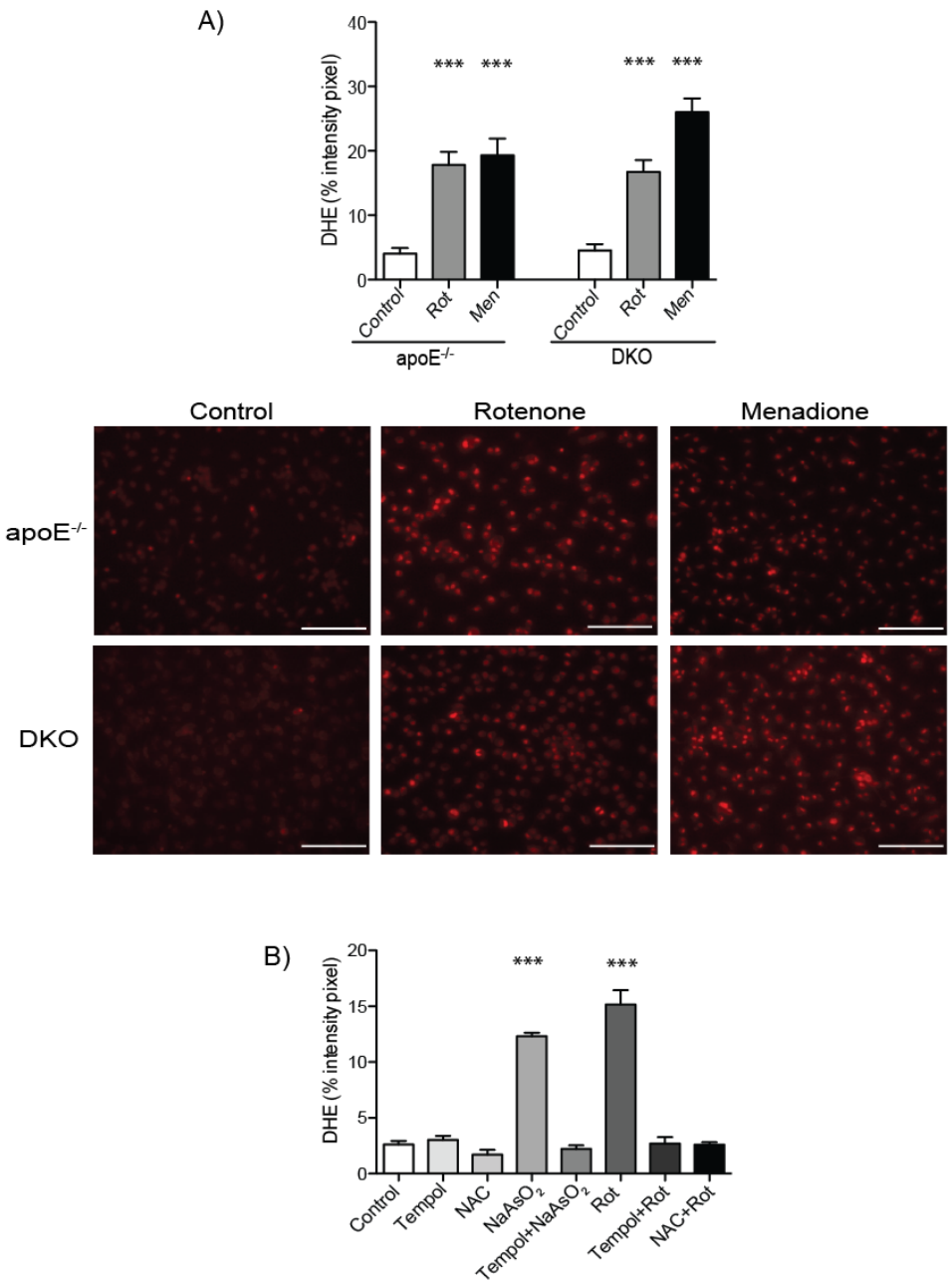

Figure S2: apoE$^{-/-}$ and DKO macrophages are responsive to reactive oxygen induction.

A) Bone marrow-derived macrophages from apoE$^{-/-}$ or DKO mice from three independent experiments, were pre-treated with 5 μM of dihydroethidium for 10 min, then cultures were exposed to 10 μM rotenone (Rot) or 200 μM menadione (Men) for a further 30 min. Cell were imaged and representatives pictures are shown. B) Bone marrow derived macrophages were pre-treated for 10 min with Tempol or N-acetylcysteine (NAC), after new media was added contained 5 μM of dihydroethidium for further 10 min, then cultures were exposed to 10 μM rotenone (Rot) or 2.3 μM (200ppb) of $NaAsO_2$ for 30 min.

A)

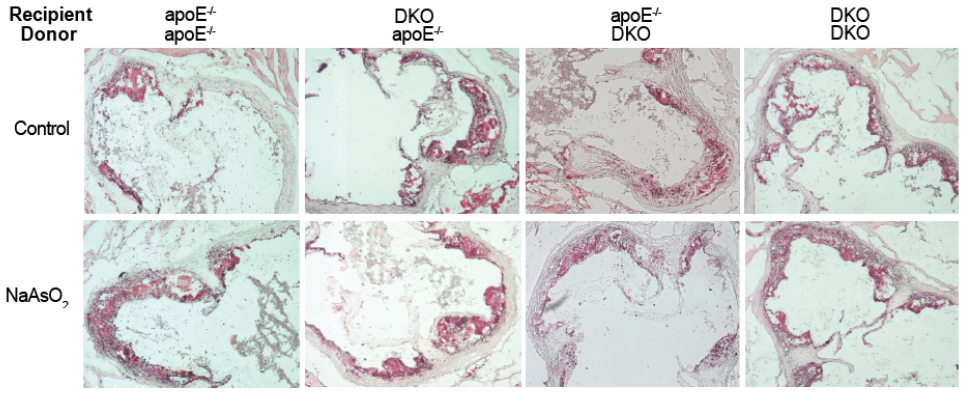

Figure S3: Representative pictures from aortic sinus of transplanted groups exposed to $NaAsO_2$ or tap water.

Five week old apoE-/- or DKO male mice were lethally irradiated and transplanted as indicate. Transplanted nine week old apoE-/- mice with apoE-/- BM, DKO mice with apoE-/- BM, apoE-/- mice with DKO BM, DKO mice with DKO BM were exposed to NaAsO2, at 200 ppb for 13 weeks or maintained on tap water. Sections of the aortic sinus were stained with oil red O.

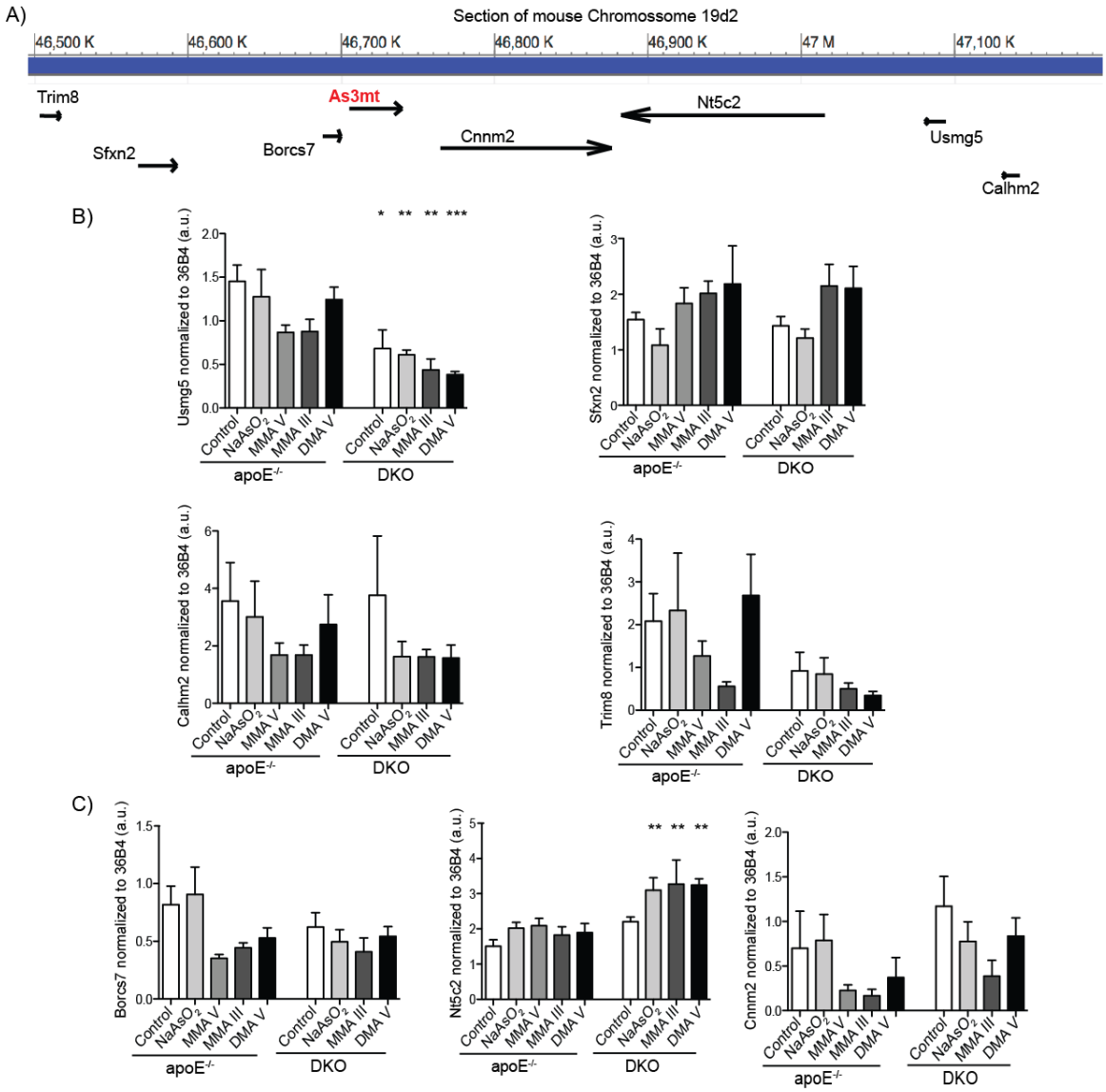

Figure S4: As3mt knockout does not alter expression of genes near As3mt at the chr 19d2.
Squematic representation of the mouse Chr 19d2 where As3mt is located and respectives analyzed genes (A). Total RNA from liver (B) and brain (C) apoE$^{-/-}$ and DKO mice (n=4-6 per group) was isolated and indicated gene expression was assessed by qPCR. Each sample was analyzed in triplicate (technical replicate) and expressed relative to the m36B4 housekeeping gene (arbitrary units). *p<0.05; **p<0.01; ***p<0.001 relative to apoE$^{-/-}$ control.

Table S1: Effect of arsenicals on plasma cholesterol, triglycerides, HDL, LDL, ALT and AST levels of apoE$^{-/-}$ and DKO mice.

Groups	Cholesterol mmol/L	Triglycerides mmol/L	HDL mmol/L	LDL mmol/L	ALT U/L	AST U/L
Control	11.4 ± 2.2	1.2 ± 0.2	1.7 ± 0.2	9.1 ± 2.0	56 ± 19	129 ± 32
$NaAsO_2$	15.3 ± 3.1	1.5 ± 0.7	1.6 ± 0.2	12.8 ± 3.0	54 ± 06	104 ± 03
MMA V	14.1 ± 1.0	1.4 ± 0.4	1.9 ± 0.1	11.4 ± 1.1	59 ± 21	132 ± 67
MMA III	11.0 ± 3.5	1.3 ± 0.1	2.1 ± 0.1	8.7 ± 2.8	62 ± 13	177 ± 48
DMA V	13.7 ± 1.4	1.4 ± 0.4	2.1 ± 0.3	10.9 ± 1.3	57 ± 16	122 ± 32
DKO Control	14.4 ± 5.1	1.3 ± 0.2	1.8 ± 0.3	11.7 ± 5.5	68 ± 34	128 ± 56
DKO $NaAsO_2$	15.7 ± 1.8	1.7 ± 0.2	2.1 ± 0.3	12.7 ± 1.6	63 ± 13	131 ± 24

No statistical difference was observed

Table S2: Complete blood count from control apoE$^{-/-}$ and DKO mice.

	apoE$^{-/-}$	**DKO**
RBC (10^6/mm^3)	10.1 ± 0.6	11.4 ± 2.2
HGB (g/dL)	16.1 ± 0.9	17.8 ± 2.8
HCT (%)	48.4 ± 2.6	48.4 ± 2.6
MCV (µm^3)	48.2 ± 1.1	47.5 ± 1.0
MCH (pg)	16.0 ± 0.4	15.6 ± 0.8
MCHC (g/dL)	33.3 ± 0.5	32.9 ± 1.0
RDW (%)	15.2 ± 0.9	15.1 ± 0.9
WBC (10^3/mm^3)	7.6 ± 2.9	6.5 ± 1.4
Lymphocyte (10^3/mm^3)	3.2 ± 1.0	3.1 ± 0.7
Monocytes (10^3/mm^3)	0.6 ± 0.2	0.6 ± 0.1
Granulocytes (10^3/mm^3)	3.9 ± 1.8	2.9 ± 0.7

No statistical differences were observed

Table S3: Primers description.

Target	Strategy	Sequence	Slope	Efficiency (%)
Borcs7	Sense	5’- AAA ATC AGG CCA GTG GTG TC -3’	-3.2	105.122
	Anti-sense	5’- GTA TCA GCG GGT CCA CTT GT -3’		
Calhm2	Sense	5’- CCT GGT GTT CCT GAC CAA GT -3’	-3.4	94.562
	Anti-sense	5’- AGA GCC ACA AAG CCA AAG AA -3		
Cnnm2	Sense	5’- AGG AGA TAG GCA CGG TCT ATA A -3	-3.3	100.606
	Anti-sense	5’- CCT TGG ATG ATG TTC AGC TCT -3’		
Nt5c2	Sense	5’- CCC TTG GCT TTG AGC TTA CT -3’	-3.0	113.80
	Anti-sense	5’- AGT CCT CTG GTA GGG AAT GTA G -3’		
Sfxn2	Sense	5’- CAT GGA GAG GCT GGA GAG AC -3	-2.9	117.644
	Anti-sense	5’- GCA CGG AGC ACT TAG AGA CC -3’		
Trim8	Sense	5’- GAC GTG GAG ATA CGG AGG AA -3	-3.0	112.524
	Anti-sense	5’- TGG TGC AGC TTT TCG TAC TG -3’		
Usgm5	Sense	5’- AAA GTG ATG GCC AAT TCC AG -3	-3.2	102.968
	Anti-sense	5’- GGC ATG GGA CTT AAC AGG TG -3’		